TECHNICAL BULLETIN, 20 JULY, 1915

MICHIGAN AGRICULTURAL COLLEGE

EXPERIMENT STATION.

BOTANICAL DEPARTMENT

EXPERIMENTS ON THE CONTROL OF THE ROOT-KNOT NEMATODE

BY

McCLINTOCK

Agent, Bureau Plant Industry.

(In charge of Ginseng Disease Investigations in Michigan.)

EAST LANSING, MICHIGAN
1915

EXPERIMENTS ON THE CONTROL OF THE ROOT-KNOT NEMATODE.*

BY J. A. MC CLINTOCK,

Agent B. P. I. in charge of Ginseng disease Investigation of Michigan.

The root-knot nematode, *Heterodera radicicola,* (Greef) Müller, has proven to be a serious pest of many crops in the southern states, and in some of the western states it has caused considerable trouble on potatoes and sugar beets. In many of the northern states, this nematode has caused serious trouble on greenhouse crops and on such intensive garden crops as ginseng.

The life history of this parasite has been worked upon by (1) Atkinson†, (6) Stone & Smith, (2) Bessey, (3 and 4) Cobb, and control measures have been worked out for greenhouse conditions by Stone & Smith; while Bessey and (5) Rolfs have recommended rotations that tend to keep the root-knot nematode more or less under control in certain field crops. Much still remained to be worked out in the way of control measures for intensive crops like truck crops, ginseng, golden seal, etc.

For an account of the complete life history of the root-knot nematode see Bessey (2). Briefly it is as follows:

In about twenty-five days after the female enters a root, she has reached sexual maturity and begun to lay eggs. These eggs which are 85-98 microns in length by 34-40 microns in thickness, are surrounded, as laid, by a gelatinous substance which holds them together in a mass. In the case of firm, woody tissues, the eggs seldom reach the surface of the knot, but produce numerous larvæ which make their way to the surface and enter new roots. Where the root-knot is in softer tissues, as those of various herbaceous plants, the eggs are often forced through the tissues and cling in small masses to the surface of the knot. The number of eggs laid by a single female varies somewhat, but counts seem to show that it is between four and five hundred. In all cases where the number of eggs used in experiments is recorded, it is on the basis of four hundred eggs per egg mass, so in most cases the number of eggs treated probably ran over rather than under the number recorded. Under favorable conditions, the eggs probably begin to hatch within a few days after being laid, thus making the complete life cycle about thirty days under favorable conditions.

*The work upon which this paper is based was carried on under a cooperative agreement between the Bureau of Plant Industry and the Michigan Experiment Station, and in somewhat different form was accepted as a thesis for the degree of Master of Science in the Department of Plant Pathology, University of Wisconsin.

†The number with the author's name refers to the number of the article referred to as listed in the bibliography at the close of this paper.

FIELD EXPERIMENTS CONDUCTED IN MICHIGAN IN 1915.

As the chief work assigned to the author in Michigan was that of experiments on the control of the root-knot disease of ginseng, it was necessary to make a general survey of the various ginseng growing sections of the state, in order to select a suitable place for establishing a field laboratory. It was finally decided that a large garden located at Muskegon, Michigan, afforded the best opportunity for carrying on field experiments so the field laboratory was located there.

In order to exclude, as far as possible, any factors which might lead to errors in the results, it was decided that the plots used for experiments should be separated from the remaining soil. To accomplish this the plots of soil, seven by ten feet in extent, were enclosed with galvanized sheet iron thirty-six inches in width. The iron was set into the soil thirty inches, leaving six inches projecting above the surface to keep any surface soil from being washed into the plots after the experiments were started. The observation of nematodes three feet down in the soil has been recorded by Bessey (2) but the difficulty of obtaining iron more than thirty-six inches wide was so great as to preclude our placing it more than thirty inches in the soil. The edges, where the sheets of iron came together, were bent, interlocked and pounded tightly together to prevent the passage of anything at such places. To exclude the possibility of soil being carried onto the plots, by various animals passing through the garden, each plot was enclosed above ground with a three foot fense of chicken-wire netting, which came down so as to overlap the six inches of sheet iron above ground.

The plots, seven in number, were alternated with two foot paths, so as to cover the greater part of a strip of soil fourteen feet wide and seventy feet long. Two beds of badly nematode infested ginseng roots had been removed from this soil the fall previous, and no crop had been on the ground since. The soil, throughout the whole garden, is a loose, sandy loam, very highly fertilized with barnyard manure, making it an ideal place for the rapid multiplication and spread of the root-knot nematode.

Although the experiments of previous workers with chemical treatments of the soil for the eradication of *Heterodera radicicola* had given chiefly negative results, it was decided that a series of such treatments should be made to test the relative value of various chemicals, with the possibility of finding one that would be both effective and practical of application.

CARBON BISULPHIDE TREATMENT.

As previous experiments by Bessey (2) had shown carbon bisulphide to be successful under field conditions, it was decided that one plot should be treated with this chemical exactly as recommended. On the afternoon of May 10, 1913, plot I was treated with carbon bisulphide as follows:—Holes were made in the soil at intervals of six to twelve inches, and varying in depth from six to eighteen inches. Liquid carbon bisulphide was poured into the holes at the rate of four ounces per square yard of soil, and the holes quickly plugged up by packing soil into them. Within half an hour after the treatment was made the air, in the vicinity of the plot, had a very strong odor of carbon bisulphide,

and the odor was still quite noticeable the next morning after the treatment. From this it was concluded that the vapor had been passing up through the soil and out into the air for a period of about eighteen hours. Lettuce, tomato and cucumber plants are among those most readily attacked by the root-knot nematode; therefore, it was decided to use these plants to determine whether or not the soil treatments had eradicated the pest. May 14, 1913, lettuce seeds were planted in the carbon bisulphide treated plot, and also in the untreated soil about the plot to serve as a check on the treatment. May 31, 1913, the seeds planted May 14th had not produced many plants so the plot was replanted with lettuce, tomato and cucumber seeds. June 16, 1913, some of the larger lettuce plants were dug from the plot and their roots found to be somewhat affected with nematodes as shown by the small knots on them. Check plants dug from the soil outside of the plot were more seriously affected than those from the treated plot, thus showing that the nematodes had been reduced in numbers, but not eradicated by this carbon bisulphide treatment.

June 30, 1913, the rest of the plants were dug from the treated soil and the roots were found to be abundantly covered with knots, showing that the nematodes were increasing in numbers in the treated soil. August 16, 1913, this plot was retreated with carbon bisulphide as before, except that the surface of the soil was sprinkled with water and covered with burlap sacks, after the treatment, in an effort to keep the carbon bisulphide vapor from passing so rapidly from the soil out into the air. August 18th the sacks were removed from the surface of the plot and lettuce seed was planted in the treated soil. September 8th some of the lettuce plants were dug and examined but no knots were found on the roots. It was impossible to get further data on this plot during the rest of the season. In the summer of 1914 the author examined lettuce and cow pea plants from this plot and found nematodes on their roots.

TOBACCO STEM TREATMENT.

The second plot was treated with tobacco stems. A wagon load of tobacco stems was collected from various cigar factories and taken to the garden. May 12, 1913, a layer of these stems over six inches in thickness was spread over all the soil of plot II. May 14, 1913, the stems were wet down with water. May 31, 1913, the layer of tobacco stems was removed from the plot and lettuce, tomato and cucumber seeds were planted in the treated soil, and also in the untreated soil outside the plot. June 16th some of the plants were dug from the treated soil and examined but no knots were found on the roots. June 30th lettuce, tomato and cucumber plants were dug from the treated soil and examined but no knots were found on their roots. August 11th, the author dug up lettuce, tomato and cucumber plants from the treated soil and found some small knots on their roots, showing that the nematodes had been reduced in numbers for a time, but not eradicated. The writer does not feel that such a treatment can be depended upon to give the desired results.

SULPHURIC ACID TREATMENT.

August 14, 1913, all of the remaining plants were dug from the second plot. The soil was then treated with a solution of one part of commercial sulphuric acid to eighty-five parts of water, using one-half gallon of this solution per square foot of soil. August 18th lettuce seed was planted in the treated soil. September 8th lettuce plants were dug from the plot and examined, but no knots were found on the roots. It was not possible to get further data on this plot during the rest of the season, but in the light of experiments performed later in the laboratory at the University of Wisconsin, it is probable that the sulphuric acid treatment did not eradicate the nematodes from the treated soil. In the summer of 1914 the writer examined plants from this plot and found root-knots on them.

FORMALDEHYDE TREATMENTS.

It was decided that the third plot should be treated with formaldehyde. In 1912 some preliminary experiments along this line were started. During April, 1913, we had an opportunity of seeing the results of these soil treatments with a solution of one part of 40% formaldehyde to fifty parts of water. These treatments had not been successful in eradicating the nematodes from the treated soil; so we decided to double the strength by using 1-25 formaldehyde (1 part of 40% formaldehyde to 25 parts of water). May 12, 1913, plot III was treated with a 1-25 solution of formaldehyde, using one gallon to a square foot of soil. May 14th lettuce and cucumber seeds were planted in the treated soil to see if they would germinate and grow before the soil was free from formaldehyde gas. May 31st no lettuce or cucumber plants had come up from the seeds planted May 14th, so the plot was planted with lettuce, cucumber and tomato seeds. June 16, 1913, some of the plants were dug and examined, but no knots were found on the roots. June 30th, the author examined lettuce, tomato and cucumbers plants, but found no knots on their roots. August 11th, he dug up and examined lettuce, tomato and cucumber plants and found some small knots on their roots. This showed that the treatment with 1-25 formaldehyde had greatly reduced, for a time, the number of nematodes in the soil, but had not eradicated them.

August 13, 1913, the writer dug up all of the remaining plants from Plot III, respaded the soil, and then raked it so as to note and remove as far as possible, any pieces of roots which had been broken from the plants in digging. The soil was then retreated with a formaldehyde solution made up at the rate of one gallon of 40% formaldehyde to sixteen gallons of water, and applied at the rate of about one gallon of solution to a square foot of soil. August 14, 1913, lettuce seed was planted on this plot to see if it would grow before the formaldehyde gas was out of the soil. September 3, 1913, no lettuce plants had developed from the seed planted August 14th; so the plot was replanted with lettuce seed. September 8, 1913, no plants were up. No further data could be taken on this treatment during the rest of the season; therefore, we came to no definite conclusion relative to the value of this treatment. During the summer of 1914 plants from this plot were examined and nematodes found in the roots.

NAPHTHALINE TREATMENT.

As naphthaline was known to be more or less of a repellant to certain insects, it was suggested that it be given a trial. The original intention was to mix flake naphthaline with the soil. As it was not possible to obtain naphthaline in the flake form at the time it was desired to start the experiment, it was necessary to use it in the form of small balls, commonly called "moth balls." May 15, 1913, plot IV was treated as follows:—Holes were made in the soil at intervals of eight inches to one foot, and varying from six to twelve inches in depth. One or two naphthaline balls were dropped into each hole and the mouths of the holes then closed with soil packed into them. About one and one-half pounds of moth balls were used on the plot. May 31, 1913, the writer planted tomato and cucumber seeds in the naphthaline treated soil. June 16, 1913, some of the plants were dug and examined and found free from root-knots. June 30, 1913, the author examined tomato and cucumber plants from plot IV and found knots on their roots. This proved that the naphthaline treatment applied would not control the root-knot nematode.

AMMONIA TREATMENT.

May 15, 1913, plot V was prepared for treatment as in plot IV, by making holes in the soil with a brass dibble. A small amount of 28% ammonia was poured into each hole, and then the holes were quickly plugged with soil. The ammonia gas soon penetrated the soil and gave the air in the vicinity of the plot a very strong odor. About three pounds of ammonia were used on this seven by ten foot plot. May 31, 1913, lettuce, tomato, and cucumber seeds were planted in the treated soil. June 16, 1913, some of the plants were dug up and knots found on their roots. This would seem to show that such an ammonia treatment had no effect on the nematodes in the treated soil. Later on in the season this plot was used for starting an experiment with ginseng roots apparently resistant to nematodes.

"BLACK LEAF 40" TREATMENT.

It had been intended to make this and the following treatment at the same time the others were made, but they were delayed because we could not get the "Black Leaf 40" and the tobacco dust at that time. "Black Leaf 40," a commercial nicotine product made by the Kentucky Tobacco Products Company, has proven quite efficient as an insecticide for certain types of insects. As a result of its success as an insecticide, it was suggested for nematodes. Directions on the sealed can recommended the use of a one-half pound can of this concentrated nicotine product to thirty-four gallons of water, for all ordinary insects. This dilution was supposed to give not less than seven hundredths of one per cent nicotine. May 31, 1913, plot VI was treated with "Black Leaf 40" solution made up according to directions, and applied to the soil at the rate of one gallon per square foot. June 2, 1913, the writer planted lettuce, tomato and cucumber seeds in the treated soil. June 30, 1913, he dug up and examined lettuce and tomato plants from plot VI and found knots on their roots, thus showing that such a dilution of "Black Leaf 40" would not control root-knot nematodes.

TOBACCO DUST TREATMENT.

Tobacco dust, made by grinding tobacco stems into a fine powder, has proven quite efficient as an insecticide; so it was determined that it should be given a trial. June 2, 1913, twenty-five pounds of tobacco dust were spread over the surface of the soil in plot VII, and thoroughly raked into the soil. The plot was then thoroughly soaked with water. Later on in the day, lettuce and tomato seeds were planted in this plot. June 30, 1913, the author dug up and examined some of the plants from plot VII and found a few small knots on their roots. August 11, 1913, he examined plants from plot VII and found knots on their roots. This would seem to show that such an application of tobacco dust as was used, would not eradicate this nematode.

KEROSENE TREATMENT.

August 11, 1913, all the plants from plot VI were dug up and the soil then treated with commercial kerosene, using ten gallons on the seven by ten foot plot. August 14, 1913,lettuce seed was planted in this plot. September 3, 1913, no plants had come up from the seed planted August 14th, so the plot was replanted with lettuce. September 8, 1913, no plants were up in this plot. No further data could be obtained on this treatment during the fall, so no conclusions could be drawn at that time. Plants from this plot were examined during the summer of 1914 and root-knots were found on them.

GASOLINE TREATMENT.

August 11, 1913, the writer dug up and removed the rest of the plants from plot VII. The plot was then divided by setting a sheet of galvanized iron thirty-six inches wide, into the soil eighteen inches. This gave two plots five by seven feet in area. One of the plots was treated with commercial gasoline, by sprinkling one gallon over the surface of the soil. The other plot was treated in a similar manner except that three gallons of gasoline were used instead of one. August 14, 1913, lettuce seed was planted in both plots. September 8, 1913, the writer dug up and examined lettuce plants from both of the gasoline treated plots and found many knots on their roots. The heavy gasoline treatment appeared to have no advantage over the light treatment, and neither seemed to inhibit the development of the nematodes seriously.

SUMMARY OF THE RESULTS OF THE FIELD TREATMENTS.

In all of the foregoing experiments the knots found on the roots of plants grown in the treated soils, were examined and dissected under a microscope to make sure of their true cause. In each case where complete data were obtainable they pointed to the conclusion that the treatment used could not be depended upon to eradicate the root-knot nematodes from the treated soil. In some cases the treatment succeeded in materially reducing the number of nematodes present in the soil, as shown by the longer periods that the plants grew in some plots before showing any knots on the roots. Treatments such as carbon bisulphide, tobacco stems or strong formaldehyde solution, which greatly reduce the number of nematodes in the soil for a month or so, might be of practical

value in the case of some rapidly growing crop, which could be matured and removed before the nematodes again became numerous enough to materially injure the plants. In the case of a slow growing crop like ginseng, which remains in the soil from four to six years, any treatment which falls short of complete eradication is worthless, because a few nematodes left in the soil would completely restock it with their kind. In the course of a year or two they would be as serious as they were before the treatment was applied. In the light of these experiments, no chemical used could be recommended for the control of root-knot nematodes; furthermore, no exact explanation could be given as to just why the treatments did not eradicate the pest.

THEORIES AS TO THE FAILURE OF THE FIELD TREATMENTS.

Various theories to explain the failure of the treatments were formulated, but none of them were backed by experimental data. It was thought possible that occasional nematodes more resistant than the average, were able to withstand the adverse conditions and that these resistant ones in time succeeded in restocking the soil, when conditions again became favorable for their growth and reproduction. A second theory was that nematodes deep down in the soil, where the treatments exerted little or no influence, came up and repopulated the treated soil after the effects of the treatments had sufficiently diminished. Another possibility was that the nematode eggs in the soil were of such a nature that they were able to resist the adverse conditions and remain dormant until favorable conditions allowed the young to come out into suitable environment for their development. As the second of the above theories did not appear to be of a nature to lend itself readily to investigation in the laboratory, it was decided to turn attention to the other two in an effort to determine what relation they might have to the failure of the soil treatments.

LABORATORY EXPERIMENTS AT MADISON, WISCONSIN.

Early in October 1913, the experiments at the field laboratory were closed for the season, and laboratory work on various phases of the subject was begun in the greenhouses and laboratories of the Department of Plant Pathology of the University of Wisconsin. For some little time after arriving at Wisconsin, efforts were confined to fitting up experiments in the greenhouses, and to getting susceptible plants started so that there might be an abundant supply of nematodes to draw from for laboratory work. Considerable time was spent in various forms of laboratory experiments before suitable technique was worked out. An effort was made to get larvae free from the plant tissues by externally sterilizing knots from lettuce roots, and then placing them on plates of sterile agar, In no case did the writer succeed in obtaining any larvae free from the knots in this way. Failing in the above method, attempts were made to get the larvae by dissecting quantities of root-knots from lettuce and other plants. It was impossible in this way to get the larvae in sufficient numbers for the treatments it was desired to make.

Next was tried removing the roundish, pearly white female nematodes from small knots on the roots of various plants. It was thought that these females might produce eggs and larvae after being transferred to

plates of agar. In most cases these females were not mature. When transferred to the plates of agar, they were soon overrun with colonies of bacteria and fungi, so that their development could not be followed. To avoid this contamination, it was tried treating the female nematodes as taken from the roots in various chemicals before transferring them to the plates of agar. Although in some cases they remained free from contamination after being transferred, in no case was success attained in getting larvæ in this way. Either their removal from the roots or the subsequent chemical treatments stopped the development of the female nematodes so that they never produced mature eggs.

The next effort was to obtain larvae by dissecting out mature or egg-laying females and egg masses from the older knots on the roots of various plants. Along with these females and egg masses were always some larvae that had hatched out within the knots from the first eggs laid by the females. Quantities of the egg masses from the root-knots were treated with various strengths of chemicals and then transferred to plates of agar. It was observed that the active larvae associated with the egg masses soon died when placed with the egg masses in the chemicals for external disinfection. This observation caused the writer to doubt the importance of our first theory; viz. that occasional larvae resisted the chemical treatments applied to the soil. The more observations that were made along this line, noting the apparent ease with which the larvae succumbed to weak chemicals, the more the writer was forced to the conclusion that the above mentioned theory was probably not the important factor in the failure of the strong chemical, soil treatments.

Other observations made about this time appeared to give more support to the theory that the mature eggs were the resistant stage. It was noted that in a few days after disinfecting mature females and egg masses, active larvae began to appear on the agar plates. As it was quite certain that no larvae had gone through the treatments alive, it was concluded that those found on the plates were larvae that had hatched from treated eggs. As a result of these observations, it was decided to discontinue for the time being, the efforts to determine the relative resistance of nematodes in the larval state, and to take up the egg resistance theory.

The first experiment which gave a possible explanation for the failure of the field experiments was begun December 23, 1913. A large quantity of mature female nematodes and egg masses was dissected from large knots on Primula roots and divided into seven lots of about an equal number of eggs. After being treated with the chemicals, the different lots were transferred to plates of sugar beet agar.

1. Placed the first lot in a solution made of one part of "Black Leaf 40" to 272 parts of water. This gave the same dilution as used in the field experiments.
2. Placed the second lot in full strength "Black Leaf 40" for a few minutes.
3. Placed the third lot in 1-1000 Hg Cl_2 for a few minutes.
4. Placed the fourth lot in 1-100 commercial 40% formaldehyde for a few minutes.
5. Placed the fifth lot in 1-10 solution of 40% formaldehyde for a few minutes.

6. Placed the sixth lot in pure liquid carbon bisulphide for a few minutes.

7. Exposed the seventh lot to carbon bisulphide vapor, over the mouth of a bottle of liquid carbon bisulphide, until all the active larvae were killed. All of these treatments were made between 3:00 and 5:00 P. M., December 23, 1913. The results of the treatments are given in table I.

TABLE I.—ACTIVE LARVAE OBSERVED.

Treatment made Dec. 23, 1913.	Dec. 24.	Dec. 25.	Dec. 26.	Dec. 27.	Dec. 28.	Dec. 31.
1. "Black Leaf 40" 1-272	Twenty	Abundant	Abundant	Abundant	Abundant	Active
2. "Black Leaf 40" full strength	None	Four	One	Two	Active	Active
3. $HgCl_2$ 1-1000	None	None	None	None	None	None
4. Formaldehyde 1-100	None	None	None	None	None	None
5. Formaldehyde 1-10	None	None	None	None	None	None
6. Carbon bisulph. liquid	Three	One	None	One	Active	Active
7. Carbon bisulph. vapor	None	One	Two	Two	Active	Active

January 2, 1914, the plates used above were all cleaned up. As the only means of detecting the presence of active larvae on the plates was by examining them under a microscope, it proved to be a rather difficult thing to locate all of the larvae after they had crawled away from the egg masses. Although the results of this experiment were not entirely conclusive either for or against the theory, it was decided to continue the work for the purpose of collecting data before coming to any definite conclusion.

EXPERIMENT II.—FORMALDEHYDE 1–100.

January 7, 1914, the writer dissected out about thirty egg masses from knots on Primula roots, and placed them in 1-100 solution of 40% formaldehyde for one and one-half hours, and then transferred them while still wet to a plate of potato agar. The results of this treatment are given in Table II.

TABLE II.—ACTIVE LARVAE OBSERVED.

Treatment Jan. 7, 1914.	Jan. 8.	Jan. 9.	Jan. 10.	Jan. 12.	Jan. 14.	Jan. 19.	Jan. 23.
Formaldehyde 1-100	None	None	One	Four	Active	Quiet	Quiet

EXPERIMENT III.—FORMALDEHYDE 1–100 AND 1–10.

January 24, 1914, a large number of egg masses were dissected out and divided into two lots. Placed the first lot in 1-100 soltuion of 40% formaldehyde for ten minutes and then transferred it, while still wet, to a plate of potato agar. Placed the second lot in 1-10 solution of 40% formaldehyde for ten minutes and then, while still wet, transferred it to a plate of potato agar. The results of this experiment are given in Table III.

TABLE III.—ACTIVE LARVAE OBSERVED.

Treatment Jan. 24, 1914.	Jan. 26.	Jan. 27.	Jan. 28.	Jan. 29.	Jan. 30.	Feb. 2.	Feb. 7.
Formaldehyde 1-100.....	None	None	None	None	None	None	None
Formaldehyde 1-10.....	None	None	None	None	None	None	None

EXPERIMENT IV.

January 26, 1914, dissected out a very large number of egg masses and divided them into eight lots. After treatment each lot was transferred to a plate of potato agar.

1. Treated the first lot in 3% hydrogen peroxide for 10 minutes.
2. Treated the second lot in 1-100 solution of 40% formaldehyde for 10 minutes.
3. Treated the third lot in 1-10 solution of 40% formaldehyde for 3 minutes.
4. Treated the fourth lot in 40% formaldehyde for 10 minutes.
5. Treated the fifth lot in 1-1000 $HgCl_2$ for 10 minutes.
6. Treated the sixth lot in saturated solution of $HgCl_2$ for a few minutes.
7. Treated the seventh lot in 20% copper sulphate for 30 minutes.
8. Treated the eighth lot in carbon bisulphide vapor for 1½ hours.

TABLE IV.—ACTIVE LARVAE OBSERVED.

Treatment Jan. 26, 1914.	Jan. 27.	Jan. 28.	Jan. 29.	Jan. 30.	Jan. 31.	Feb. 1.	Feb. 3.	Feb. 4.	Feb. 5.
1. Peroxide 3%.....	15	Abund.	Abund.	Abund.	Abund.	Abund.	Abund.	Abund.	Abund.
2. Formaldehyde 1-100.....	None	None	None	None	None	None	None	None	None
3. Formaldehyde 1-10.....	None	None	None	None	None	None	None	None	None
4. Formaldehyde 40%.....	None	None	None	None	None	None	None	None	None
5. $HgCl_2$ 1-1000.....	None	None	None	One	One	One	One	One	One
6. $HgCl_2$ Sat. Sol.....	None	None	None	None	None	None	None	None	None
7. $CuSO_4$ 20%.....	None	None	None	None	None	None	None	None	None
8. Carbon bisulphide.....	None	None	None	None	None	None	None	None	None

EXPERIMENT V.

February 2, 1914, dissected out a large number of egg masses from knots on tomato roots and divided them into eight lots. After treatment each lot was transferred to a plate of lima bean agar.

1. Treated the first lot in 3% hydrogen peroxide for 30 minutes.
2. Treated the second lot in 1-100 solution of 40% formaldehyde for 15 minutes.
3. Treated the third lot in 1-10 solution of 40% formaldehyde for 15 minutes.
4. Treated the fourth lot in 40% formaldehyde, full strength for 15 minutes.
5. Treated the fifth lot in 1-1000 Hg Cl_2 for 15 minutes.
6. Treated the sixth lot in a saturated solution of Hg Cl_2 for 30 minutes.
7. Treated the seventh lot in 20% copper sulphate for forty-five minutes.

8. Treated the eighth lot in carbon bisulphide vapor for 17 hours. The results of this experiment are given in Table V.

TABLE V.—ACTIVE LARVAE OBSERVED.

Treatment Feb. 2, 1914.	Feb. 3.	Feb. 4.	Feb. 5.	Feb. 6.	Feb. 7.	Feb. 9.	Feb. 10.
1. Peroxide 3%.....	7	70	Many	Many	Many	Quiet	Appeared dead
2. Formaldehyde 1-100..	None	1	4	21	Abundant	Abundant	Abundant
3. Formaldehyde 1-10...	None	None	None	None	None	None	None
4. Formaldehyde 40%..	None	None	None	None	None	None	None
5. $HgCl_2$ 1-1000.....	1	1	1	1	1	None	None
6. $HgCl_2$ sat. sol.....	None	None	None	None	None	None	None
7. $CuSO_4$ 20%.....	None	2	2	7	Abundant	Quiet	Appeared dead
8. Carbon bisul. vapor.	None	None	None	None	None	None	None

February 10, 1914, all of the plates of the above series were cleaned up except the 1-100 solution of 40% formaldehyde. This plate was observed from time to time to see how long the larvae would remain alive. March 30, 1914, active larvae were observed.

EXPERIMENT VI.

February 28, 1914, dissected out many egg masses and divided them into four lots. After treatment, each lot was transferred to a plate of potato agar, and the plates sealed shut with paraffin.

1. Treated the first lot by rinsing it in boiled tap water.

2. Treated the second lot in 1-100 solution of 40% formaldehyde for 3 minutes.

3. Treated the third lot in 1-25 solution of 40% formaldehyde for 3 minutes.

4. Treated the fourth lot in 1-10 solution of 40% formaldehyde for 3 minutes. The results of these experiments are given in Table VI.

TABLE VI.—ACTIVE LARVAE OBSERVED.

Treatment Feb. 28, 1914.	March 1.	March 2.	March 3.	March 4.	March 5.
1. Boiled water.....	Larvae present	Larvae present	Larvae present	Larvae present	Larvae present
2. Formaldehyde 1-100.....	None	None	None	None	None
3. Formaldehyde 1-25.....	None	None	None	None	None
4. Formaldehyde 1-10.....	None	None	None	None	None

As the conditions under which these laboratory experiments were being conducted differed greatly from those in the field, it was concluded that possibly one or more of these factors was influencing the results, thus making the laboratory results very inconclusive. Perhaps the most obvious difference between the laboratory and the field experiments was the surroundings of the nematodes and eggs after treatment. In the field experiments the eggs were in the soil where plenty of air and moisture could get to them to dilute the various chemicals applied. In the laboratory experiments the eggs were enclosed in petri dishes which tended to keep out the air and moisture, and to keep in the volatile chemicals. This was especially true when the plates were sealed with paraffin. If petri dishes did act as an important factor in causing the chemicals to in-

hibit nematode egg development, it was thought that this influence might be even greater if the containing vessel were smaller and more tightly sealed. To test this point, we decided to run a series of treatments in culture slides instead of petri dishes.

EXPERIMENT VII.

March 7, 1914, dissected out a large number of egg masses from infested roots and divided them into eleven lots of about 3,000 eggs each. After treatment each lot was transferred to a cover glass and sealed down over a culture slide.

1, 2 and 3. The first three lots were rinsed in sterile water and served as checks on the treatments.

4. Treated the fourth lot in 3% hydrogen peroxide for a short time.

5. Treated the fifth lot in 1-100 solution of 40% formaldehyde for a short time.

6. Treated the sixth lot in 1-25 solution of 40% formaldehyde for a short time.

7. Treated the seventh lot in 1-10 solution of 40% formaldehyde for a short time.

8. Treated the eighth lot in 40% formaldehyde for a short time.

9. Treated the ninth lot in 1-1000 Hg Cl_2 for a short time.

10. Treated the tenth lot in pure liquid carbon bisulphide for a short time.

11. Placed the eleventh lot on a cover-glass and exposed it to carbon bisulphide vapor for ten minutes.

The results of these experiments are given in table VII.

TABLE VII.—ACTIVE LARVAE OBSERVED.

Treatment March 7, 1914.	March 8.	March 9.	March 10.	March 11.	March 12.	March 13.
1. Sterile water	Very many	Very many	Very many	Very many	Very many	Discontinued
2. Sterile water	Very many	Very many	Very many	Very many	Very many	Discontinued
3. Sterile water	Very many	Very many	Very many	Very many	Very many	Discontinued
4. Peroxide 3%	Very many	Very many	All quiet	Quiet	Quiet	Discontinued
5. Formaldehyde 1-100	14	None	None	None	None	Discontinued
6. Formaldehyde 1-25	None	None	None	None	None	Discontinued
7. Formaldehyde 1-10	None	None	None	None	None	Discontinued
8. Formaldehyde 40%	None	None	None	None	None	Discontinued
9. $HgCl_2$ 1-1000	Very many	Very many	Used for a	culturing ex	periment.	
10. Carbon bisul. liq	None	None	None	None	None	Discontinued
11. Carbon bisul. vapor	One	None	Two	None	None	Discontinued

With the exception of the 1-1000 Hg. Cl_2 treatment, the various chemicals all produced more or less of an inhibitory effect on the eggs. And, even where the eggs did hatch, as in the 1-100 formaldehyde and the carbon bisulphide vapor treatment, the larvæ soon died. From this latter fact, it would appear that chemicals were still present in the sealed culture slides to such an extent as to be intolerable to the larvae. The large numbers of larvae that hatched from eggs in the check slides would seem to show that the chemicals were exerting a great influence. This and the previous experiments with closed vessels pointed quite strongly toward the surroundings as an important factor in our laboratory experiments.

In order to see whether the exception, in the case of the 1-1000 Hg Cl_2

treatment of our last experiment, was a normal one, it was decided to repeat this part of the experiment.

EXPERIMENT VIII.

March 9, 1914, dissected out a number of egg masses and placed them in 1-1000 Hg Cl_2 for two minutes. The egg masses were then transferred to drops of agar on four cover glasses, and sealed down over four culture slides. The results of this experiment are given in Table VIII.

TABLE VIII.—ACTIVE LARVAE FOUND.

Treatment March 9, 1914.	March 10.	March 11.	March 12.	March 13.
$HgCl_2$ 1-1000	Very many	Very many	Abundant	All quiet
$HgCl_2$ 1-1000	Very many	Abundant	Abundant	All quiet
$HgCl_2$ 1-1000	Very many	Used for c	ulturing expe	riment.
$HgCl_2$ 1-1000	Very many	Used for c	ulturing expe	riment.

The results of this experiment appeared to verify those of the previous experiment, and to show that a short exposure to 1-1000 Hg Cl_2 does not keep the nematode eggs from hatching.

As it now seemed probable that the various chemicals exerted an inhibitory effect in closed vessels, it was decided that data should next be obtained on treatments conducted under conditions more nearly like those present in the soil of the field experiments. To accomplish this, the egg masses were placed after treatment in tubes of agar, closed only by cotton plugs. A synthetic agar medium was prepared by adding one and two-tenths per cent of powdered agar and one per cent of glucose to Pfeffer's nutrient plant solution. This on being sterilized and cooled, solidified into a solid medium, through which one could easily see. A number of tubes of this medium were prepared, using about ten c. c. to a tube.

EXPERIMENT IX.

March 14, 1914, dissected out a large number of egg masses from root-knots and divided them into twelve lots. After treatment, each lot was transferred to a tube of synthetic agar while it was still wet with the solution in which it had been treated.

1. Placed the first lot in 1-100 solution of 40% formaldehyde for a few minutes.
2. Placed the second lot in 1-25 solution of 40% formaldehyde for a few minutes.
3. Placed the third lot in 1-10 solution of 40% formaldehyde for a few minutes.
4. Placed the fourth lot in full strength 40% formaldehyde for a few minutes.
5. Placed the fifth lot in 1-500 Hg Cl_2 for a few minutes.
6. Placed the sixth lot in 1-200 Hg Cl_2 for a few minutes.
7. Placed the seventh lot in 1-100 Hg Cl_2 for a few minutes.
8. Placed the eighth lot in full strength "Black Leaf 40" for a short time.
9. Placed the ninth lot in full strength lime-sulfur, 31-32° B., for a few minutes.

10. Placed the tenth lot in the same lime-sulfur as the ninth lot. After being in this solution for a few minutes, the egg masses were transferred to a drop of synthetic agar on a cover glass and sealed down over a culture slide instead of being placed in a tube of synthetic agar.

11. Placed the eleventh lot in full strength commercial sulphuric acid for a few minutes, and then transferred it to a slide of agar.

12. Placed the twelfth lot in 1-100 sulphuric acid for a few minutes. The results of this experiment are given in Table IX.

TABLE IX.—ACTIVE LARVAE OBSERVED.

Treatment March 14, 1914.	Vessel No. of eggs used.	Mar. 15.	Mar. 16.	Mar. 17.	Mar. 18.	Mar. 20.	Mar. 22.	Mar. 24.
1. Formaldehyde 1-100	Tube 2000	Abund.	Abund.	Abund.	Abund.	Abund.	Abund.	Abund.
2. Formaldehyde 1-25	Tube 3000	None	Abund.	Abund.	Abund.	Abund.	Abund.	None active
3. Formaldehyde 1-10	Tube 3000	One	Abund.	Abund.	Abund.	Very many	Very many	Very many
4. Formaldehyde 40%	Tube 3000	None	None	None	.....	Two	Abund.	Very many
5. $HgCl_2$ 1-500	Tube 3500	Very many	Very many	Very many	.....	Very many	Very many	Many
6. $HgCl_2$ 1-200	Tube 2000	Very many	Very many	Very many	.....	Very many	Very many	.....
7. $HgCl_2$ 1-100	Tube 2000	Many	Many	Many	.....	Many	Many	Many
8. "Blk Lf. 40" full strength	Tube 3000	Very many	Very many	Very many	.....	Very many	Very many	None active
9. Lime sul. full strength	Tube 3000	One	Abund.	.....	.....	Abund.	.....	None active
10. Lime sul. full strength	Slide 3000	None	None	None	None	None	.....	None
11. H_2SO_4 full strength	Slide 3000	None	None	None	None	None	.....	None
12. H_2SO_4 1-100	Slide 3000	None	None	None	None	None	.....	None

The results of this experiment as a whole, are so different from those of any of the previous tests that one is strengthened in the opinion that the containing vessel must have been a factor influencing the results. From the table, it will be noted that the treatments which allowed little or no hatching were those where sealed slides were used instead of test tubes. The lime-sulfur treatments give a good comparison of the difference due to the vessel used, because all other conditions were about the same. By referring to Experiment IX, it will be seen that the egg masses were all subjected to the same treatment with lime-sulfur, and then 3,000 eggs were placed in a tube of synthetic agar and 3,000 were placed on a drop of synthetic agar and sealed down over a hollow slide. Abundant larvae hatched from the eggs in the tube, while none hatched from an equal number of eggs in a culture slide. The amount of agar used might be considered as an important factor in bringing out the difference in the result, but by referring to the earlier experiments, it will be seen that where one tube, ten c. c., of agar was spread out over a plate, there was about the same inhibitory effect as in the sealed slide with one drop of agar.

This comparison of the type of vessel used was made for the purpose of trying to account for the great difference between the results obtained in the early experiments and those of later trials. In the early ones was

noted a large per cent of inhibition of hatching, while in the later experiments, there was almost no inhibition from treatments with the same, and also with much stronger chemicals. In order to get further data on the inhibitory power of sulphuric acid, as compared with the other chemicals used, it was decided to repeat this treatment, using tubes of agar instead of slides.

EXPERIMENT X.

March 16, 1914, dissected out a large number of egg masses and divided them into three lots. These lots were treated for a few minutes in 1-100 H_2SO_4, 1-50H_2SO_4, and 1-25 H_2SO_4, respectively, and then transferred, while still wet, to three tubes of synthetic agar. The results of this experiment are recorded in Table X.

TABLE X.—ACTIVE LARVAE OBSERVED.

Treatment March 16, 1914.	Number of eggs used.	March 17.	March 19.	March 20.
H_2SO_4 1-100	5000	Abundant	Abundant	Abundant
H_2SO_4 1-50	5000	Abundant	Very many	Very many
H_2SO_4 1-25	5000	Very many	Very many	Very many

The results of this experiment would seem to show that even quite strong sulphuric acid solutions have no more of an inhibiting power than the other chemicals used, when the conditions are made the same.

EXPERIMENT XI.

This experiment was made for the purpose of determining the relative value of carbon bisulphide. March 20, 1914, about twelve thousand eggs were dissected out from knots on lettuce, squash and tomato roots, and divided into two lots of six thousand eggs each. The first lot was placed in pure liquid carbon bisulphide for a few minutes, and then transferred, while still wet, to a tube of synthetic agar. The second lot was placed on a hollow slide, and the slide was then inverted over the mouth of a bottle of carbon bisulphide. After being exposed to the strong vapor for twenty minutes, the egg masses were transferred from the slide to a tube of synthetic agar. The results of this experiment are given in Table XI.

TABLE XI.—ACTIVE LARVAE OBSERVED.

Treatment March 20, 1914.	Number of eggs used.	March 21.	March 22.	March 25.
Liquid Carbon bisulphide	6000	Twelve	Abundant	Abundant
Vaporized Carbon bisulphide	6000	Many	Many	Abundant

From these results it was concluded that treatments with carbon bisulphide, such as the above, do not materially inhibit the hatching of the nematode eggs.

In all of those experiments with tubes of media, the synthetic agar was used. This medium gave a substratum as nearly like that of the soil as could be obtained and still have it clear enough to examine under a microscope, to determine the presence of larvæ. One of the questions that come to mind in the use of this synthetic medium, was that of the possible effect of the various salts of the medium on the chemicals used in the treatments. Did not these various salts tend to act on certain of the chemicals so as to make them lose their power to inhibit the hatching of the nematode eggs? If this occurred in the synthetic medium, one might conceive that it also occurred in the soil and thus might partially account for the failure of certain field experiments. To obtain data on this point, it was decided to run a series of treatments on a medium as free from these various nutrient salts as could be obtained. The best medium that could be devised for this purpose was a plain agar made of distilled water stiffened up with 1.5% of powdered agar. A number of tubes of this medium were made, using about ten c. c. to a tube.

EXPERIMENT XII.

March 21, 1914, a large quantity of eggs was dissected from knots on roots of lettuce and squash plants, and divided into twelve lots. After treatment each lot was transferred to a tube of plain agar.

1. Placed the first lot, of 3000 eggs, in liquid carbon bisulphide for a few minutes.
2. Placed the second lot, of 5000 eggs, on a cover glass and inverted it over the mouth of a bottle of carbon bisulphide for six and one-quarter hours. On removal from the bottle the egg masses were found to be very dry and hard.
3. Placed the third lot, of 4000 eggs, in 1-100 $HgCl_2$ for a few minutes.
4. Placed the fourth lot, of 3000 eggs, in a 20% solution of $CuSO_4$ for a few minutes.
5. Placed the fifth lot, of 5000 eggs, in full strength lime-sulfur 31 to 32° B. for a few minutes.
6. Placed the sixth lot, of 3000 eggs, in 1-100 H_2SO_4 for a few minutes.
7. Placed the seventh lot, of 3000 eggs, in 1-50 H_2SO_4 for a few minutes.
8. Placed the eighth lot, of 3000 eggs, in 1-25 H_2SO_4 for a few minutes.
9. Placed the ninth lot, of 3000 eggs, in full strength H_2SO_4 for a few minutes.
10. Placed the tenth lot, of 2000 eggs, in 1-100 solution of 40% formaldehyde for a few minutes.
11. Placed the eleventh lot, of 3000 eggs, in 1-25 solution of 40% formaldehyde for a few minutes.
12. Placed the twelfth lot, of 3000 eggs, in 1-10 solution of 400% formaldehyde for a few minutes.

The results of this experiment are given in Table XII.

TABLE XII.—ACTIVE LARVAE OBSERVED.

Treatment March 21, 1914.	No. of eggs used.	March 22.	March 23.	March 24.	March 25.	March 26.	March 27.
1. Liq. carbon bisulphide	3000	None	None	None	One	One	Abundant
2. Vapor carbon bisulphide	5000	None	None	None	None	None	None
3. $HgCl_2$ 1-100	4000	None	None	None	Two	Many	Many
4. $CuSO_4$ 20% solution	3000	None	None	Abundant	Abundant	Many	Many
5. Lime-sulf. full strength	5000	None	None	None	None	Abundant	Many
6. H_2SO_4 1-100	3000	Abundant	Abundant	Used for cu	lturing experi	ment.	
7. H_2SO_4 1-50	3000	Very many	Very many	Used for cu	lturing experi	ment.	
8. H_2SO_4 1-25	3000	None	None	None	None	Abundant	Abundant
9. H_2SO_4 full strength	3000	None	None	None	None	None	None
10. Formaldehyde 1-100	2000	None	Abundant	Very many	Very many	Very many	Very many
11. Formaldehyde 1-25	3000	None	One	Two	Abundant	Very many	Very many
12. Formaldehyde 1-10	3000	None	None	Abundant	Abundant	Many	Many

With the exception of the carbon bisulphide vapor treatment for six and one-quarter hours, and the full strength sulphuric acid treatment, it will be observed that the results of the various treatments of this experiment correspond with those of the previous experiments, where tubes of agar were used. As there was no more inhibition of hatching in this last experiment, where plain agar was used, than in the other tests, where synthetic agar was employed, it would seem that the various salts of the synthetic agar did not reduce the inhibitory powers of the chemicals used in the treatments.

EXPERIMENT XIII.

It was thought advisable to repeat the carbon bisulphide treatments to get more definite data than the previous experiments had supplied. March 23, 1914, dissected a number of egg masses from knots on lettuce roots, and divided them into three lots. After treatment, each lot was transferred to a tube of synthetic agar while still wet.

1. Placed the first lot in pure liquid carbon bisulphide for a few minutes.
2. Placed the second lot in carbon bisulphide vapor for one hour.
3. Exposed the third lot to strong carbon bisulphide vapor for sixteen and one-half hours.

The results of this experiment are given in Table XIII.

TABLE XIII.—ACTIVE LARVAE OBSERVED.

Treatment March 23, 1914.	No. of eggs used.	Mar. 24.	Mar. 25.	Mar. 26.	Mar. 27.	Mar. 31.	April 3.
1. Liquid Carbon Bisulphide	5000	Many	Many	Many	Many	Discontinued	Discontinued
2. Carbon Bisulphide vapor	6000	One	Abund.	Abund.	Abund.	Many	Many
3. Carbon Bisulphide vapor 16½ hours	6000	None	None	None	None	None	None

In the case of the liquid treatment and the vapor treatment for one hour, it will be observed that abundant larvæ subsequently hatched from the eggs, while none hatched from the eggs exposed to the carbon bisulphide vapor for sixteen and one-half hours. From this it would ap-

pear that the longer exposure to the carbon bisulphide vapor was the only cause of this difference. It was observed, however, that the egg masses dipped in liquid carbon bisulphide, and those exposed to the vapor for one hour did not become dried out as did those exposed to the vapor for sixteen and one-half hours. This observation caused us to raise the question as to what effect the loss of water might have on the results.

EXPERIMENT XIV.

March 24, 1914, dissected out a quantity of egg masses from knots on lettuce roots and placed them in a culture slide. The slide was then placed on the window sill at room temperature and left there until the next day. March 25, 1914, after eighteen and one-half hours of such exposure, the egg masses which had dried down to small, hard lumps, were transferred to a tube of synthetic agar. The results of this experiment are given in Table XIV.

TABLE XIV.—ACTIVE LARVAE OBSERVED.

Treatment March 24-25, 1914.	No. of eggs used.	March 26.	March 27.	March 28.	March 31.	April 3.
Dried at room temp. for 18½ hours.....	10,000	None	None	None	None	None

As the egg masses had no surface disinfection, they were not free from viable fungus spores, so by March 31, 1914, the surface of the medium was so over-run by fungi that the egg masses could no longer be distinctly seen. The result of this experiment is the same as that of the long exposure to carbon bisulphide vapor; so it would appear that the drying which the egg masses underwent in the vapor treatment, might have had some inhibitory effect.

In looking over the data on some of the earlier experiments, it was observed that in many cases the writer had subjected the egg masses to the chemicals for longer periods of time than in the later experiments. It was thought that this longer exposure, ten to thirty minutes, might have caused the difference in results. To test this point it was decided to run a series of treatments in which the egg masses should be exposed for thirty minutes or more to the chemical used, before being transferred to the tubes of agar.

EXPERIMENT XV.

March 30, 1914, a large number of egg masses were dissected from knots on squash and tomato roots and divided into seven lots. After treatment, each lot was transferred to a tube of synthetic agar.

1. Placed the first lot, of 5000 eggs, in 31-32° B. lime-sulfur for thirty minutes.
2. Placed the second lot, of 5000 eggs, in full strength "Black Leaf 40" for thirty minutes.
3. Placed the third lot, of 3000 eggs, in 1-100 solution of 40% formaldehyde for forty-five minutes.

4. Placed the fourth lot, of 5000 eggs, in 1-25 solution of 40% formaldehyde for forty-five minutes.
5. Placed the fifth lot, of 5000 eggs, in 1-10 solution of 40% formaldehyde for thirty minutes.
6. Placed the sixth lot, of 5000 eggs, in 20% $CuSO_4$ for thirty minutes.
7. Placed the seventh lot, of 5000 eggs, in 1-50 H_2SO_4 for thirty minutes.

The results of this experiment are given in Table XV.

TABLE XV.—ACTIVE LARVAE OBSERVED.

Treatments March 30. 1914.	No. of eggs used.	Mar. 31.	April 1.	April 2.	April 3.	April 4.	April 5.	April 6.	April 7.
1. Lime-sulfr. 31-32° B.	5000	None	None	One	None	None	Ten	Abundant	Abundant
2. "Black Lf. 40" full strength.....	5000	None	None	None	None	None	Two	None active	None active
3. Formaldehyde 1-100.	3000	None	One	Abund.	Abund.	Abund.	Abund.	Abundant	Abundant
4. Formaldehyde 1-25..	5000	None	None	None	Abund.	Abund.	Abund.	Abundant	Abundant
5. Formaldehyde 1-10..	5000	None	None	None	None	None	12	Abundant	Abundant
6. $CuSO_4$ 20%.....	5000	None	None	None	7	Abund.	Abund.	Abundant	Abundant
7. H_2SO_4 1-50.....	5000	5	Abund.	Abund.	Abund.	Abund.	Abund.	Abundant	Discontinued

In the case of the lime-sulfur and the "Black Leaf 40" treatments, the smaller number of active larvœ recorded was due to the fact that the medium was so clouded by the treatments that larvæ could not be distinguished unless they crawled up on the sides of the tubes above the substratum. From the result of this experiment, it would seem that the longer exposure to the chemicals does not affect the ability of the eggs to hatch.

In attempting to subject the eggs to the same conditions that they are under in the actual soil treatments, it would appear that they should be exposed to the chemical for only a short time. In soil treatments we cannot conceive of the eggs being subjected to the chemical except just during the period when the solution is passing down through the soil directly after being applied at the surface. The various vapor treatments are exceptions to the above statement because they exert an influence throughout the time they are present in the soil.

SUMMARY OF THE LABORATORY EXPERIMENTS.

Although the number of experiments conducted so far is not great, the number of eggs used in each experiment was so large that we feel justified in drawing certain conclusions from the results. The results of the earlier experiments were somewhat erratic; but taken as a whole, this series leads one to conclude that the chemicals used cannot be depended upon to control the root-knot nematode, because they do not completely inhibit the hatching of the eggs. We do not feel justified in concluding that this is the only reason for the failure of the chemical treatments in the field experiments, because we have not as yet collected sufficient data on the other theories to draw any definite conclusions regarding them. Stone and Smith (6) found that chemicals failed to inhibit the

development of the eggs of certain free-living nematodes. The results of the above-mentioned workers, together with those obtained by the writer with the eggs of the parasitic nematode, *Heterodera radicicola,* indicate that further work along this line may establish the fact that the other parasitic nematodes will be able to survive soil treatments with chemicals for the same reason, namely, that the egg stage in the life cycle is resistant to the chemicals.

DATA ON STEAM STERILIZATION OF SOIL.

During the summer of 1913 it had been intended to conduct experiments on the control of the root-knot nematode by steam sterilization, but it was impossible to get a steam boiler for a sufficient length of time to prepare complete experiments. The writer did succeed in getting a boiler for one day, and so was able to collect some data relative to the depth to which the soil could be heated in a given length of time with equipment such as he had. The method and results of this work as given below, will give an idea of the value of steam, but these results are not sufficient to serve as a basis for definite recommendations. A pan ten feet six inches long, three feet wide, and six inches deep, was used in this work. Steam was furnished by a ten horsepower boiler kept at a pressure of seventy pounds. The soil to be treated was first thoroughly spaded up to loosen it to facilitate the penetration of the steam.

In order to get data on the depth to which the steam penetrated, potatoes were placed in the soil at three places, viz., at the end of the pan where the steam entered, at the opposite end, and at the middle. Holes eighteen inches deep were dug and stakes set in them to mark the location of the potatoes. One potato was placed at the bottom of each hole, eighteen inches below the surface, and the soil was shoveled in until the holes were twelve inches deep. At this depth potatoes were placed in each hole and soil shoveled in until the holes were six inches deep. Potatoes were placed in each hole at this depth, and then the holes were filled until they were but three inches deep. Potatoes were placed at this depth and the holes were then filled up until the soil was level over the whole plot. The pan was then connected up with the boiler and the steam turned on. In about fifteen minutes the steam began to lift the pan out of the soil so that steam escaped from under its edges. To avoid this loss of steam, the pan was weighted down with scrap iron. After thirty minutes of treatment the steam was shut off and the pan removed from the soil. The potatoes in the hole farthest from where the steam entered the pan, were dug first. The potatoes at three inches, six inches, and twelve inches below the surface were well cooked throughout and the one at eighteen inches below the surface was very hot. Next, the potatoes in the middle of the plot were dug up, and all found to be well cooked, even the one eighteen inches below the surface. It was next attempted to dig the potatoes at the end of the plot where the steam entered, but the steam had gouged out such a hole in the soil that it was impossible to locate all of them. The two that were found were well cooked. The soil treated is a very fertile clay loam, with a stiff clay subsoil at about twenty-four inches below the surface.

This steam experiment was next repeated on an adjoining plot of soil exactly as before, except that the steam at eighty pounds pressure was run into the pan for fifteen minutes instead of thirty minutes. At the end of the period the pan was removed and the potatoes were dug. The potato at three inches below the surface was thoroughly cooked. Potatoes at six and twelve inches below the surface were partly cooked. The potato at eighteen inches below the surface was very hot. Of these two treatments, the thirty minute steaming was the better, as judged by the condition of the potatoes. Where the potatoes were well cooked, it is quite reasonable to suppose that the nematodes were killed, but the writer is not certain of this point.*

ACKNOWLEDGMENTS.

The writer is indebted to Dr. E. A. Bessey, Botanist, Michigan Agricultural Experiment Station, for many suggestions and much helpful advice.

To Dr. L. R. Jones, Plant Pathologist, Wisconsin Agricultural Experiment Station, the writer is indebted for much helpful criticism.

LITERATURE.

The literature cited here is chiefly that which has a bearing on this particular problem. For the more general literature on the subject, see the bibliography by Bessey (2).

Atkinson, George F.

(1) A preliminary report upon the life history and metamorphoses of a root-gall nematode. Heterodera radicicola (Greef) Muhl., and the injuries caused by it upon the roots of various plants. Alamaba Polytechic Institute Bul. N. S., No. 9, 1889.

Bessey, Ernst A.

(2) Root-knot and its control. Bureau of Plant Industry Bul. 217:7-81. 1911.

Cobb, N. A.

(3) Root-gall. The Agricultural Gazette of New South Wales. Vol. 12, No. 9, pp. 1041-1052. 1901.

(4) The internal structure of the gall-worm. The Agricultural Gazette of New South Wales, Sidney, Vol. 13, No. 10, pp. 1031-1033. 1902.

Rolfs, P. H.

(5) Tomato diseases. Florida Agricultural Experiment Station, Bul. 91; pp. 15-34. 1907.

Stone, George E. and Smith, Ralph E.

(6) Nematode worms. Hatch Experiment Station of the Massachusetts Agricultural College, Bul. 55; pp. 2-67. 1898.

*NOTE: Observations made by E. A. Bessey in the summer of 1915 on these and similarly treated adjacent lots of formerly badly infected soil show the total absence of root-knot nematodes both one and two years after the steam treatment.